Lecture Notes Professor Chris Rudd October 24, 2015

The Lecture will outline the key mediators and stages of signal transduction with references to their connection to different immunopathologies.

Topics

1) T-cell and B-cell receptors (TCR/BCR)
2) Microcluster formation and the immunological synapse (IS)
3) CD4/CD8-p56lck and ZAP-70 (and autoimmunity)
4) Phosphatases CD45 and PTPN22 (and autoimmunity)
5) Adaptors (LAT, SLP-76, ADAP, SKAP1)- SLP-76 and autoimmunity
6) Calcium mobilisation (IP3, STIM1 and CRAC)

During antigen/peptide presentation by dendritic cells (DC), T-cells adhere to DCs leading to the ligation of antigen receptors that in turn activate protein kinases that phosphorylate an array of substrates. DCs present foreign or self-peptide in the context of members of the major histocompatibility complex (MHC). Serine/threonine phosphorylation is the most common form of phosphorylation that involves the activation of extracellular-signal-regulated kinases (ERKs), p38 and c-Jun N-terminal kinase (JNK). By contrast, tyrosine phosphorylation is far less common, but is crucially linked to the phosphorylation of **immunoreceptor-based tyrosine activation motifs (ITAMs)** on the TCR and BCR complexes. In T-cells, the concomitant binding of CD4 and CD8 to conserved sites on their respective class II and I antigens brings the signalling complexes, **CD4–p56*lck* and CD8–p56*lck*,** into the proximity with MHC leading to the phosphorylation of the cytoplasmic domains of the TCRζ and CD3 chains. p56*lck* is an immune cell specific protein-tyrosine kinase (PTK) that phosphorylates ITAMs needed for the binding of two Src-homology 2 (SH2) domains of other PTK called zeta-associated protein 70 (**ZAP-70**). SH2 domains are discrete modules that bind to phosphotyrosine-based motifs. These receptors and kinases oligomerise to form **microclusters** that can be visualised by microscopy at the interface between T-cells and antigen-presenting cells. The interface is termed the **immunological synapse (IS)**. Mutations in the antigen receptor and ZAP-70 lead to opposite clinical outcomes such as SCID-like immuno-deficiencies or the development of **collagen induced arthritis (CIA).** Mutation in the SH2C domain of ZAP-70 (that prevents binding to the zeta chain of the TCR complex) causes a defect in negative selection in the thymus leading to the development of CIA.

Unlike kinases, abundant phosphatases act to suppress activation of T-cells. Two prime examples are **CD45 and PTPN22**. While $p56^{lck}$ is regulated by phosphorylation

of two sites: inhibitory Y-505 and stimulatory Y-394. Y-505 and Y-394 are dephosphorylated by CD45. The kinase is normally held in a closed, inactive conformation by an interaction between the phosphorylated tyrosine and the internal SH2 domain of the kinase. De-phosphorylation by CD45 unfolds and both activates suppresses the kinase. A single site polymorphism (snp) in PTPN22 links a **multiple autoimmune diseases** that include type 1 diabetes, rheumatoid arthritis, juvenile arthritis, systemic lupus erythematosus and Graves disease. These autoimmune disorders had previously been thought to be unconnected. An understanding of signalling was needed to show that they were interconnected in a common pathway.

Substrates of the kinases, $p56^{lck}$ and ZAP-70 include a group of proteins called **molecular scaffolds or adaptors**. These proteins lack enzymatic activities, and instead are characterised by the presence of binding modules and sites for the formation of large complexes called **signallosomes**. It is thought that these complexes act like **molecular switches** that integrate signals needed for difference functions.

One central adaptor is the transmembrane adaptor LAT (linker for activation of T cells) that regulates phospholipase (PLCγ1) activation and Ca2+ mobilisation.

Paradoxically, LAT-deficient Jurkat T cells show defects in TCR-induced Ca^{2+} mobilization/ ***LAT signalosome*** (LAT/GADS/SLP-76) activation of phospholipase Cγ is also needed for the function of transcription factor termed nuclear factor for the activation of T-cell (NFAT) for cytokine gene transcription. The N-terminus of the adaptor SLP-76 acts to bring a kinase (ITK) to the LAT signalosome to phosphorylate and activate phospholipase Cγ1 (PLCγ1). PLCγ1 hydrolyses phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)*P* 2], generating the second messengers diacylglycerol (DAG) inositol (1,4,5)-trisphosphate [Ins(1,4,5)*P* 3] (also termed **IP3**). IP-3 binds to and opens $InsP_3$ receptors ($InsP_3Rs$) in endoplasmic reticulum (ER) that releases Ca^{2+} from intracellular Ca^{2+} stores. A subsequent decrease of Ca^{2+} in ER causes **stromal interaction molecule 1 (STIM1)** redistribution into puncta which co-localise with **ORAI1-containing calcium-release-activated calcium (CRAC) channels** for their activation. Ca^{2+} influx though CRAC channels and elevated intracellular Ca^{2+} activate Ca^{2+}-dependent enzymes, such as calcineurin, and thereby transcription factors such as NFAT. Calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK506) potently block T-cell activation, and are in clinical use to prevent transplant rejection and severe forms of autoimmune disease. A new generation of drugs to prevent transplant

rejection will include inhibitors of STIM1 and CRAC channels.

Paradoxically, mutation of the PLCγ1 binding site in LAT leads to an extreme form of autoimmunity. Cells from these mutant mice are mostly T-helper 2 (Th2) cells that secrete IL-4 and IL-10. This leads to greatly enlarged B-cell follicles and a massive increase in IgG1 secretion.

ADAP-SKAP1 signalosome (SLP-76/ADAP/SKAP1). The other end of the SLP-76 adaptor binds to another adaptor termed ADAP (adhesion and degranulation-promoting adaptor protein) and SKAP1 (src kinase-associated phosphoprotein1; also SKAP-55, src kinase-associated phosphoprotein-55) that in turn is coupled to the SKAP1, an effector that controls the activation of integrin for the binding to T-cells to DCs.

References

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